

LIFE for LASCA INTERNATIONAL CONGRESS

Book of abstracts and summaries



*LIFE SAVING LASCA Urgent measure to conserve nearly extinct species
Protochondrostoma genei*

LIFE16 NAT/SI/000644

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Preface

This document encompasses abstracts and summaries of presentations from the online international LIFE for LASCA congress, which took place on December 2, 2022. The purpose of the congress was to disseminate the LIFE for LASCA project results and achievements, as well as to share knowledge and experience with participants and other related projects. The focus of the LIFE for LASCA project is to improve the conservation status of a cyprinid fish species, the South European nase (*Protochondrostoma genei*) or more commonly named Lasca. Therefore, the general focus of the meeting was this species, as well as conservation topics of other endangered fish species, which inhabit similar or the same river systems and are protected under the Annex II/V of Habitats Directive. The congress attended 121 participants. We were very pleased to have the opportunity to view and listen to 13 presentations from various contributing authors, which presented their projects and scientific works regarding status and conservation of delicate fish species and other relevant issues in fisheries conservation and management.

The congress was divided into three following sessions/parts based on topics considered:

1. Lasca conservation in the entire species distribution range
2. Life for Lasca project: Implementation and project results with future prospects
3. Endangered game fish species conservation status



1. LASCA CONSERVATION IN THE ENTIRE SPECIES DISTRIBUTION RANGE

Lasca conservation status in northern part of Italy

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The first certain observations of *Protochondrostoma genei* in Friuli Venezia Giulia (Northeast Italy) were reported by Tellini (1895), for rivers Sile and Natisone. Other reports confirmed the presence of *P. genei* in the Isonzo River, Livenza River, Stella River, Tagliamento River and in the Corno Stream (Gridelli, 1936; Tortonese, 1970; Flego, 1972; D'Este, 1978). More recently, specimens were captured in the Meschio River (Livenza basin) and in the Arzino Stream (Tagliamento basin) (Stoch et al., 1992).

In Friuli Venezia Giulia, *P. genei* was always observed with low densities and populations rarely well structured, but in the last years captures have further decreased, mainly due to water pollution, flow regulation and habitat degradation. Moreover, in the Isonzo River Basin the species is threatened by the presence of allochthonous *Chondrostoma nasus*, due to trophic habitat competition.

Recently, an abundant and well-structured *P. genei* population was observed and studied in the Chiarò Creek (Corno Stream Basin), during a study carried about as a result of an agreement and collaboration between University of Trieste and the Polytechnic University of Turin. The aim was to characterize preferential habitats for *P. genei* life-history stages and identifying pivotal factors to protect the species. The study is still ongoing, but collected data provides useful information, due to the importance of the proper habitat identification, especially after the heavy drought occurred in the last year. Finally, as the *C. nasus* nase was collected in the Chiarò Creek, an eradication project started in 2022, to reduce its population in the creek, where juveniles migrate from downstream watercourses in search for suitable habitats.



Lasca in Po River basin (status and conservation)

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Lasca (*Protochondrostoma genei*) has almost completely disappeared along the middle and lower course of the Po River, mostly due to the interactions with the alien species introduced and the river fragmentation that limits reproductive migration. Strong regression throughout Piedmont and Lombardy, especially in the middle and lower stretch of the main waterways (Adda, Ticino). Currently, the state of conservation of the Lasca in the Po river basin is rather critical, with an estimated range of distribution in the origin areas that varies roughly between 25% and 60% compared to the reference one. The main threats that have caused the rapid decline in the Po River basin are represented by river fragmentation, which hinders the long migratory movements of the species and the banalization of the habitats of the rivers valley floodplains. Lasca migrates upstream during the reproductive period to areas with more turbulent and oxygenated waters. The presence of obstacles to such migrations or the absence of suitable areas for reproduction therefore, has an impact on the survival of the species. The presence of barriers or the simplification of habitats negatively affects this species, affecting it in a crucial phase of its life cycle. Frequent floods and predation of cormorants, which had an exponential demographic increase in recent decades and the progressive diffusion and affirmation of invasive exotic fish species also, have a strong impact, primarily the Wels catfish (*Silurus glanis*).



Conservation of endangered species of the upper Po River; including Lasca

LIFE MINNOW; Small fish, small streams, big challenges: conservation of endangered species in tributaries of the upper Po River*

Fenoglio S.#

*Reference: LIFE21-NAT-IT-LIFE-Minnow/101074559 | Acronym: LIFE21-NAT-IT-LIFE Minnow.

<https://webgate.ec.europa.eu/life/publicWebsite/project/details/101074559>

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The project objective is to prevent the decline of six freshwater fishes included in the Annex II of Habitat Directive: Po brook lamprey (*Lethenteron zanandreae*), South European nase (*Protochondrostoma genei*), Italian nase (*Chondrostoma soetta*), Italian loach (*Sabanejewia larvata*), Italian rifle dace (*Telestes muticellus*) and Sculpin (*Cottus gobio*). *T. muticellus* is evaluated in the conservation status as U1 Unfavourable-Inadequate and the other five as U2 Unfavourable-Bad. The specific objectives are (1) restoration of longitudinal connectivity for 122 km of waterways currently fragmented by 16 barriers; (2) habitat improvement in 13 strategic points; (3) restocking of the population of five species supported by fish farms; (4) alien species control and reduction in 440 km within Natura 2000 targeted sites in Piedmont Region in northern part of the Po River basin; (5) construction of allochthonous biomass reuse trough an agreement with a pet food company. A wide approach of the stakeholders is foreseen, with active participation in the conservation actions (fishermen, volunteers, students, citizens), as well as with involvement of local community to increase the value of natural resources via the Citizen Science approach. For the long-term conservation of the species, the project will draft and approve specific conservation measures; Guidelines for river continuity will provide updated data to improve measures of the Po River Basin Management Plan of Water framework Directive. Project results will be transferred and replicated by the implementation of stakeholder groups, who will carry out networking activities in Italy, Spain and France in areas that are facing similar threats for similar species. The project contributes to the LIFE work programme implementation by applying the best practice considering Natura 2000 sites and other adjacent areas.



Lasca *Protochondrostoma genei* (Bonaparte, 1839) conservation status and measures at Apennine area, Italy.

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The dramatic decline of the Lasca, *Protochondrostoma genei* populations within its native range was observed also in the Apennine area; it could be related to the multiple anthropogenic stressors to which the species appears to be particularly sensitive, as river fragmentation, water pollution and biological invasions. In the 1960s the species was unintentionally introduced into the Tiber river basin (Tuscany-Latium ichthyogeographic district), where Lasca is currently present in large and abundant populations. The aims of our study were to: i) analyze the conservation status of Lasca in some Adriatic basins of the central Apennines, within the southern limit of its original range, using fish data collected by electrofishing from 2007 to 2021; ii) analyze the dispersal pattern and assess the possible negative impacts of Lasca on native endemic species inhabiting the Tiber river basin, such as the Etruscan chub (*Squalius lucumonis*) and the South Italian roach (*Sarmarutilus rubilio*), using data collected from 2000 to 2016.

Our results showed that the Lasca distribution in the Adriatic basins was fragmented and the residual populations were characterized by low density values. In the Tiber basin, the species acclimatized rapidly following its introduction, spreading in the hydrographic network, where an overall increase of Lasca population abundance has been observed over time. In the new colonized environments, *P. genei* became a member of the fish community without showing a particular invasiveness, even if long-term negative impacts cannot be excluded, as evidenced by the lower values of Relative Weight calculated for *S. rubilio* and *S. lucumonis* in co-occurrence with the Lasca.

The preliminary results of a pilot project confirmed the hypothesis of a competitive interaction between the Lasca and the native *S. lucumonis*. The pilot project also showed the practical application, for conservation purposes, of using the eradicated *P. genei* individuals for reintroduction programs in sites where the species has become locally extinct, or to support declining populations within the native range of the species.

The information provided by the present study, concerning the distribution, abundance, biology and ecology of Lasca, could be a basis to plan appropriate management measures, aimed at the recovery of the species within its original area.



2. LIFE FOR LASCA PROJECT: IMPLEMENTATION AND RESULTS WITH FUTURE PROSPECTS

Lasca (*Protochondrostoma genei*) conservation status and measures in Slovenia

LIFE for LASCA; LIFE SAVING LASCA Urgent measure to conserve nearly extinct species
*Protochondrostoma genei**

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*Reference: LIFE16 NAT/SI/000644 | Acronym: LIFE for LASCA | www.lifeforlasca.eu

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Native Lasca (*Protochondrostoma genei*) specimens in Slovenia are present only in a small region of the country named Goriška Brda. This population was, and still is very small and endangered (vulnerable). We estimate that total number of specimens does not exceed 200 specimens, occupying less than 6 km of watercourses. In Slovenia, historically, Lasca specimens were present also in Vipava valley (Natura 2000 site), from where the species has disappeared mainly due to alien species presence and habitat destruction and loss.

Life for LASCA project started in October 2017 with the aim to improve the species conservation status in Slovenia. The final goal of the project was the reintroduction of Lasca species into Natura 2000 site Dolina Vipave [SI3000226]. During the project, two partners successfully collaborated Parco Ticino from Italy and Fisheries Research Institute from Slovenia. Local help was also crucial for project implementation. Special thanks goes to local Angling clubs Ajdovščina and Renče and to other enthusiastic individuals who provided local knowhow and extensive help in the field. The project is in the final phase, ending in the year 2022. The project major success is successful repopulation of Natura 2000 site Dolina Vipave [SI 3000226]. We have released 136.052 specimens into three selected watercourses. The first release was in October 2019. Released specimens have dispersed along the entire suitable habitat within the streams and they survived all extreme weather conditions in the wild, including low temperatures during winters and high during summer as well as, floods and extreme droughts. Even more, we have confirmed that released Lasca specimens spawn in the wild. This success is a major step towards self-maintaining capabilities of wild populations. In June 2021, we have found released Lasca specimens in stream Jovšček, just before spawning. Mature females were full of eggs; males exhibited breeding colors and tubercles on top of their heads. Furthermore, in October 2022, in stream Močilnik, we found 4-5 cm long Lasca specimens representing offspring from 2022. These specimens could not arrive from anywhere else but from the 2022 spawning in the wild.

The project started with Lasca stock from Italy, which was transported to Slovenia for breeding in captivity. Transferring knowledge from LIFE CON FLU PO project on Lasca breeding was crucial for the success. Lasca breeders in Slovenia performed four successful spawning seasons in captivity, producing 41.000 offspring. Parco Ticino (Italy) significantly supported Lasca production with their well-established captive breeding practice and produced more than 95.000 offspring. Offspring was intended for reintroduction into nature and as well as for a genetic backup for the future. Prior to the wild release of specimens, careful selection of release sites (N=3) and sufficient control of threats were established. It is important to specify that the "3" release sites were three streams, where fish were released at many micro locations. These micro locations were selected based on seasonal weather and hydrological conditions, as well as species requirements; we selected the most comparable watercourse sections to those in Goriška Brda where native Lasca population is still



present in Slovenia. We compared fish communities and habitats, performed electrofishing sampling, river habitat surveys, compared water flow dynamics, riparian vegetation structure and shading, substrate composition and sediment supply. Using temperature loggers, we compared water temperature annual dynamics. Utilizing a flow meter, we created stream profile features, which enabled us to analyze water depth, riverbed width and stream discharge. This resulted in the selection of three Lasca release sites in Natura 2000, named Jovšček, Močilnik and Ozlenšček. Besides the selection of release sites, the very important step was the definition of pressures and threats to Lasca and their reduction and control. Intensive habitat destructions in second half of the 20th century and the presence of the alien Common nase (*Chondrostoma nasus*) caused Lasca disappearance in Natura 2000 site in the past. These two major pressures still exist today, but to lesser extent. Hydrological works are less extensive and are under the control of Institute of the Republic of Slovenia for Nature Conservation (IRSNC). In addition, public awareness towards nature protection has been increasing. However, in the future, we still need to continue with strong awareness activities and act as an expert support to IRSNC. However, during the project we noticed and recorded that habitat is still being disrupted by anti-flood construction interventions and removal of riparian vegetation. The faster the water drains from the watercourse, the earlier drought occurs. This is not a very promising disclosure, since global warming events are becoming more frequent and extreme. Presence of alien species also played a crucial role in Lasca disappearance in Vipava valley (Povž, 1983, 1986 a, b). Young common nase compete with Lasca for a habitat and food. During the project, we removed more than 10.000 alien specimens. We repressed common nase at crucial points to prevent the intrusion of a large number of alien specimen into Lasca release sites. Additionally, we decreased spawning potential by removing larger specimens on spawning and feeding grounds. Despite of this, for the future, the only possibility is the coexistence of both species, since the complete eradication of alien species is not possible without a significant influence on other species including endangered ones. Therefore, our future task is to monitor and help Lasca in establishing a strong population and a balance with alien species. The final delivery of the project is the Action plan for Lasca conservation. Long-term goal of the plan is to achieve or propose coordinated species management throughout its entire distribution range with emphasis towards habitat protection, wild population reinforcement and invasive species control. All the best practices from relevant projects will be included as support for further management of the species. In spite of very successful projects, which gave Lasca good prospects for the future, we must recognize that the general status of the species is still poor. Specimen's finds outside the operational project areas are rare and numbers are declining, which calls for fast action. Unfortunately, required measures cannot be applied until we gather sufficient available funds. However, a popular saying provides hope, "where there is a will, there is a way".



Life for Lasca project* awareness activities and project results dissemination

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Parco Lombardo della Valle del Ticino played a crucial role in the Life for Lasca project in terms of knowledge transferability. Parco's extensive experience in cyprinid fish breeding and awareness activities gained through prior projects provided the Slovenian leading beneficiary, the Fisheries Research Institute of Slovenia (FRIS) with much needed support. Successful international communication and knowledge transfer among organizations with similar goals is of great importance in achieving species conservation goals, such as Lasca breeding, reintroduction and management. In order to insure species conservation for the long run, public awareness activities are important. This ensures the education of youth and other common public about the species importance, protection and threats, as well as creates a nature "care factor" amongst people in general.

The most important function of public awareness activities in species conservation projects is to reinforce good results. Life program gives special attention to these dissemination actions. Life for Lasca project also complemented this by organizing annual events, which involved anglers, angling associations, students, young researchers, and other common local public. This was commonly implemented by the assistance of Park rangers and volunteers. It is imperative to emphasize that these events were difficult to achieve, because of the long lasting COVID pandemic. Parco established special strategies to complete project objectives during this difficult phase of the project. Events, which involved live socializing, had to be reconfigured and adapted to health safety rules. Target groups such as, families, primary and secondary schools participated in nature hikes and slow rafting expeditions. Additionally, a significant number ($N \geq 60$) of news bulletins were regularly updated on the Parco web page and other social media. A nature photo contest was organized and promoted in the "La rivista della natura" magazine. Thousands of species information brochures were distributed among schools. Between the years 2021 – 2022, Lasca and other fish conservation projects were presented at relevant events in Italy such as, Caselle Landi traditional exhibitions and university training workshops. These LIFE events create and strengthen bonds amongst the scientific and public community and increase the interest in nature protection.



3. ENDANGERED GAME FISH SPECIES CONSERVATION STATUS

Genetics as a tool in endangered species conservation

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All living things are coded by four nucleotides, stringed in unique sequence. Molecular genetics reveals the information sequences harbor and investigates the differences between individuals/populations/species. Differences emerge as changes in the sequence (mutations), that don't influence the individual/population (neutral mutations) or those that have potentially very deterministic effects on survival of the individual/population (adaptive mutations) - due to changes in fitness. With neutral markers we are tracing two main population dynamics events; (1) Separation of populations (allopatric speciation) and (2) reconnection of previously separated populations (hybridization). These events leave traces of genetic signature in investigated genetic loci/genes/genomes, as molecular geneticist gather this information and retrace past events. Depending on the question in mind, we use the appropriate tools for the job, as single nuclear or mitochondrial marker with slow mutation rate, for first taxa screenings and putative species discovery, while for discovery of population structure fast mutating markers, such as microsatellites or certain nuclear SNPs are more appropriate. As next generation sequencing technologies became more accessible, in depth screening of individuals and populations with high number of neutral and adaptive markers is at our disposal. With screening of adaptive markers, we are now able to determine and quantify, locally adapted genes, which persist in populations within specific environments.

With the use of ddRAD sequencing of Adriatic grayling specimens, we were able to obtain genotypes with +100k loci from museum specimens. These originated before massive anthropogenic 20th century translocations of perialpine individuals, into the rivers of northern Adriatic basin. We observe, that there are still two populations, where anthropogenic introgression is not present, representing the last of non-introgressed *T. aeliani*. These populations could serve as potential donors for translocation of Adriatic grayling into the Soča river, where population of Adriatic grayling is effectively non-existent. In order to reintroduce grayling into the Soča river, we checked adaptive compatibility between Sesia and Soča river. Relatively small adaptive differences between these two populations were revealed, with more prominent local adaptations being; (1) oxygen and (2) food availability, (3) adaptation to spawning time as well as adaptation to (4) local pathogens. As these could have deterministic effect on the success of translocation, we conclude that in the event of potential translocation, natural selection on the translocated population and their offspring, should be allowed to take place. This would allow the population to adapt to local environment without interference, due to fishing and quite different selection present in hatchery environment.



Barbel conservation status in Italy

Conservation and management of *Barbus meridionalis* and *Barbus plebejus* in the Emilian tributaries of Po River*

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In Italy, barbel fish species are amongst the most endangered and can be used as a tool to preserve rivers, since these species are considered as bio indicators of water quality. Climate changes have lately been the most devastating threats to these species due to water loss, especially in the Apennine region. These events combined with the ongoing pressures to fish, such as loss of river connectivity and alien species presence highly contribute to the reduction and fragmentation of fish assemblages. Italian populations of Common Barbel (*Barbus plebejus*) and Southern Barbel (*Barbus meridionalis*) are increasingly declining, as evidenced by the update of the IUCN Red List, where both species are listed as “vulnerable” and “endangered”. Constant decline of populations created the need for interventions, both at environmental level and artificial propagation. During the Life Barbie project, the main objective was the conservation and recovery of native populations of the two mentioned barbel species in the Emilian tributaries of the Po River in the Rete Natura 2000 site. The detailed study of the density and distribution of populations, as well as an analysis of threats established the basis for further actions. Repopulation/reinforcements in connection with environmental suitability and composition of fish communities were applied. Specific in situ (reduction of biodiversity loss/defragmentation) and ex situ (breeding practices) interventions have been implemented. Furthermore, control of non-native invasive species and conservational guidelines also plays a significant role for a sustainable management of these species.

One of the biggest challenges during the project were the artificial reproduction procedures of the barbels. Gametes from spawners in the wild were obtained from specimens caught by electrofishing. Hormonal induction had to be applied to individuals for successful striping of the eggs and accomplishment of further reproduction phases. This was especially difficult with the canine barbels, due a small size of specimens, which deliver few eggs and small quantities of sperm. This is indicative to the Apennine Rivers, where populations rely on a few small individuals when compared to Alpine regions with populations that are more productive. It took 2 spawning seasons to achieve positive results and reach the declared project goal to produce 60.000 fry juveniles for population reinforcements/repopulations.

The integral environmental part of the project and a core action was the construction of seven different fish passages on the Enza River in northern Italy. This great endeavor required many coordination dialogs with the public organization in charge of the river management. Initially the Italian law prohibited any hydrological interventions due to potential flooding danger. However, after successful communication with the organization regarding river ecology, an agreement was reached. The goal of the project was achieved, which resulted in 30 km of restored river connectivity. Coordination arguments lasted for over a year, and in the end provided a very important experience in nature conservation. After concluded construction works, fisheries monitoring actions followed. Many fish species were recorded using fish passes during migration runs. Interesting to point out was that Lasca was also amongst the sampled fish species. Before the construction of fish passes, Lasca were not reported in this area.



Status and conservation of the European eel (*Anguilla anguilla*) in Friuli Venezia Giulia Region

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The management measures of the European eel stock in Friuli Venezia Giulia Region are presented in the framework of the European eel management plan in Italy (EU Regulation n. 1100/2007). The activities aimed at the conservation of the Eel followed mainly three steps: restocking, evaluations of the status of the stock, fish bans. The restocking activity mostly with Eels at the elver stage has been carried out in different environmental situations, and took the majority of the conservation efforts. However, it gave the most relevant results, even though on a local scale: the monitoring activity in the Brancolo river basin confirmed that the restocking allowed the recovering of a healthy stock, possibly composed by released and immigrated Eels. The extensive monitoring activity allowed to verify the possible effects of the restocking as well as to evaluate the status of the population in the coastal area. Here we found a patchy distribution and abundance of the Eel, however in a context of a good availability of habitats and wetlands suitable for Eel life. The next steps of the local management activities will regard the possible restoration of the riverine connectivity. Finally, all the restrictions to fishery activities have always been shared with the local fishermen cooperatives and associations. We strongly believe that any conservation activity should involve the stakeholders with a bottom-up approach, to gain the most effective strategy for the conservation of this ever-fascinating animal.



Marble trout (*Salmo marmoratus* Cuvier 1829) conservation status and management in Northern Italy: results from online survey

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CNR – IRSA engages in a wide spectrum of water resource management and aquatic ecosystem conservation research. Through various projects (IdroLIFE, LifePREDATOR), fisheries conservation and management topics are researched and applied, with the aim to preserve endangered and threatened fish species. Regarding endangered species, marble trout management and level of its conservation in Northern Italy we performed an online survey. This large salmonid with a dominant “marble pattern” phenotype is subendemic in Italy and very important to conservationists, researchers and anglers. These combined immense interests in the species generate a high level of cultural and economic importance.

The species morphological taxon is consistent with molecular phylogenetic and phylogeographic reconstructions, but threatened by the loss of native genomes and hybridization with brown trout (*Salmo trutta*). Habitat destruction and overfishing negatively affect the species longevity as well. Marble trout is included in the IUCN Red List as “least concern”. However, at the national level it is listed as critically endangered. These problems, combined with the lack of a comprehensive overview of management and conservation approaches, pose uncertainties for the species survival in the future.

The online survey questions specifically targeted 19 fisheries managers from different regions in Italy and Switzerland, covering 24 main rivers. The most concerning results were regarding the species status, for which 63% answered a decreasing trend and no managers reported an increase. The most exposed factors negatively affecting the species conservation status were the loss of native genome and habitat degradation. Additionally, predation by cormorants was also addressed as a significant threat. However, due to the lack of a scientific approach in quantification of the impact, predation by piscivorous birds needs more meristic research. In terms of angling measures and regulations, 50% answered that their area managed includes only 10% of no “kill zone”. In general, very few sanctuaries are mentioned. Minimum harvest limit of 40 cm was included in the majority (50%) of answers; with one manager implementing a maximum size limit of 60 cm. Supportive, breeding is practiced by 89% of managers, and 58% state that stocking is necessary, because the natural reproduction is not sufficient to sustain a healthy population. Larvae and juveniles are the main life stages for stocking with the origin of specimens mostly arriving from local closed and semi-closed productions. Selection of spawners by phenotype occurs in 47% of answers, whereas genetic analysis are used by 35% of managers. The most commonly used diagnostic markers are mtDNA + LDH + microsatellites (34%) and mtDNA + microsatellites (22%), followed by nuclear microsatellite and LDH diagnostics. Large variation in management approaches reveals the need for standardization (ex. genetic analysis protocols), which in turn could help to reduce misinformation (ex. mtDNA = species) of anglers and public administrators.



Marble trout (*Salmo marmoratus*) and Adriatic grayling (*Thymallus aeliani*) conservation status and measures in North-western Italy

Conservation and management of marble trout and adriatic grayling in the Dora Baltea catchment*

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*Reference: LIFE20 NAT/IT/001341 | Acronym: LIFE GrayMarble | www.lifegraysmarble.eu

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The LIFE GrayMarble project aims to improve the local conservation status of two freshwater fish species in danger of extinction in Italy, the Marble trout (*Salmo marmoratus*) and the Adriatic grayling (*Thymallus aeliani*). The project area coincides with the original range of the species within the hydrographic basin of the Dora Baltea, in northwestern Italy, involving provinces of the Metropolitan City of Turin (Piedmont) and Aosta (Valle d'Aosta). The Marble trout is included in Annex II of the Habitats Directive (92/43/EEC). According to the IUCN criteria, it is classified in Italy as Critically Endangered, with decreasing populations throughout the entire distribution area. The Adriatic grayling was recognized as an independent species only recently and was immediately classified, by the IUCN as Critically Endangered, with very few relict populations in the entire original range of distribution. Main threats to these species include hybridization, lack of knowledge on the residual populations' status, alterations of river longitudinal connectivity and natural hydrologic regimes. Water abstractions and insufficient minimum viable flows pose a serious threat, especially when coupled with climate change. Lack of suitable hatcheries and management guidelines with specific regulations additionally create difficulties in management of both fish species and their habitats.

Previous research, conducted since 2007 by the project partners, has highlighted the dramatic decline of these two salmonids in the entire northwestern sector of their distribution, especially as regards to the catchment area of the Dora Baltea. Genetic analyzes on 200 wild reproducers of Marble trout returned very high levels of hybridization (93%) with the Atlantic brown trout (*Salmo trutta*). Furthermore, along 450 km of hydrographic network potentially suitable for hosting this endemism, only two populations, in no more than 20 km of river, resulted in possession of a high degree of purity, a situation that bodes well for the achievement of the project objectives. According to the latest update of the IUCN Red List in regards to the Adriatic grayling, it is considered extinct in almost all of its original range, with the exception of only two residual populations in Piedmont. In the case of the Dora Baltea River, several individuals are still present, distributed along a river stretch not exceeding 20 km, but whose genetics have yet to be clarified and defined.

Project results are expected to improve the conservation status of both species. Identification of "Evolutionary Significant Units" and "Management Units" will provide different source populations for artificial reproduction. Improvement and modernization of infrastructure for rearing genetically identified breeders will assist in the development of purebred stocks for the reinforcement of residual natural populations and reintroductions. Removal of barriers obstructing fish migrations will reconnect the core area stretch of the river Dora Baltea, including a Natura 2000 site. Prevention of interfertile trans-Alpine trout and grayling releases in the original range of the target species will reduce hybridization potential. Enhancement of species conservation policies and Natura 2000 sites, by promoting protocols for fish management will be prepared. Involvement of stakeholders and the viability study development for transferring the project to rivers Sesia and Tanaro (Italy) and River Nestos (Eastern Macedonia and Thrace - Greece) will additionally strengthen project results and transfer knowledge amongst relevant organizations.



Restoring marble trout (*Salmo marmoratus*) genes in Soča (Isonzo) River basin

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Historically, within the Soča River basin in Slovenia, the main threat to marble trout (*Salmo marmoratus*) populations were anthropogenic introductions of the non-native brown trout (*Salmo trutta*). First evidence of brown trout stocking dates back to 1906. However, current review of historic forest management records of the local "Idrija mercury mine" suggested that brown trout stocking was already practiced fifty years before. It was revealed that there were small-improvised hatcheries along tributaries of the Idrija River (tributary of the Soča River). Much later, genetic analysis also suggested that brown trout was already introduced in this area in that time. Stocking continued through both world wars. Fish communities in rivers were depleted by the front line. This gave the introduced fish more space, less competition and the opportunity to adapt and disperse along the river network. Brown trout stocking in the Adriatic Sea basin continued until the year 1996, when it was banned by the Slovenian legislature.

Serious actions had to be acknowledged in order to preserve the marble trout. Artificial propagation was a crucial step towards achieving the goal. Despite different interests, several fish farms/hatcheries in the Adriatic region existed where actions could be taken already in the 1930s; hatchery/fish in Kobarid, Lepena, Ilirska Bistrica and Solkan. In the 1990s fish farms/hatcheries in Modrej – Tolmin and Idrija (Kanomlja) followed. Challenges such as, broodstock establishment, defining sources of specimens, stocking and regulations strategies all had to be addressed in order to reach common goals. In the beginning, wild spawners were caught with no or just phenotypical selection and sometimes stripped in the wild or kept in reservoirs to reach maturity. Later, broodstocks were established with first phenotypical selections. Broodstock establishments based on genetic selection followed according to the genetic tools available in fisheries management at the time. First strategies were stocking nursery streams with larvae and 0+ juveniles, which were electrofished after a 2-year cycle and moved into the main river. After, stocking was conducted directly into the main river with early life stages, eye eggs and 0+ juveniles. Main stocking periods were in late winter to early summer. Revised marble trout angling regulations were put in place: change of technique from spinning to fly fishing (catch&release, barbless hook), shortened season to protect spawning grounds and increased fishing for brown trout and hybrids.

Groundwork was established and repopulation projects with scientific support were launched. In the beginning of 1980s, Fisheries Research Institute of Slovenia and Coastal Angling Clubs Association found a rare remaining pure marble trout population in the stream Zadlaščica. This was the source of specimens for the first closed cycle marble trout artificial propagation. In 1993, the project for the rehabilitation of marble trout in Slovenia was launched in collaboration with Biological station Tour del Valat and Slovenian angling clubs Tolmin and Idrija. An Action plan was devised and activities are still ongoing in order to preserve marble trout for the future. Genetic analyses during repopulation projects have been evolving and providing scientific assistance, which is still crucial for success. Research helped to define the genetic status before the repopulation projects, testing isolated populations and extracting marble trout genes from hybrid zones for a better genetic diversity. Evaluation of conservation measures in Volarja stream included DNA samples extractions for more than 20 years. It was estimated, that from 67% of brown trout genes at the beginning, repopulation efforts reduced the alien genes to only 15%. In conclusion, with good conservation practices, knowledge and sufficient habitat, any small conservation management group around the world can protect their fish biodiversity.



Marble trout (*Salmo marmoratus*) and Adriatic grayling (*Thymallus aeliani*) conservation and trends in waters under Fisheries Research Institute of Slovenia management

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In the Adriatic basin of Slovenia, Fisheries Research Institute of Slovenia (FRIS) manages the upper stretch of Soča River with tributaries. Carefully managed angling is practiced only in designated districts of the Soča River and two tributaries Lepena and Koritnica. Springs and headwaters of the main river and of all tributaries (nursery streams) are sanctuaries protected under conservation measures listed in Fisheries management plans (FMPs). The native game fish species of this area are marble trout (*Salmo marmoratus*) and Adriatic grayling (*Thymallus aeliani*). In the last century, both species underwent a sharp decline due to hybridization with genes from the Danube River basin. Marble trout native genes were successfully saved with implementation of the 20-year Action plan and relevant conservation measures. In spite of this, FRIS electrofishing surveys show that the marble trout abundance numbers are decreasing and further conservation actions are crucial for preservation of the species in the future. On the other hand, fisheries surveys indicate that the Adriatic grayling in Soča river basin is in a very critical status in terms of fragmented populations, low numbers and high levels of alien genes. This creates the need for urgent attention and action as soon as possible. Furthermore, in recent times both species are under constant threats from river fragmentation, unsustainable tourism, piscivorous animals (cormorants and lately otter), loss of spawning grounds, poaching and climate change including rising temperatures and extreme weather fluctuations.

In Slovenia, the Adriatic grayling populations are very small with a high percentage of Danube genes. The most concerning situation is that the only backup broodstock was lost, due to an unfortunate disease, diminishing the only source for wild Adriatic grayling reinforcement. Since then, mainly due to rarity of specimens, Slovenia despite the effort did not succeed to establish a new broodstock from existing wild populations. This means a high threat to the species. According to genetic analysis, all the extant Adriatic grayling have the entire genome evenly mixed with the Danube genes, displacing about 3/4 of native genome (Snoj, 2020). Therefore, it is not possible to find individuals with above-average native genetic content leading to the conclusion that the restoration of the species cannot be successful using grayling from the Soča river basin. Therefore, to save the native species, Slovenia urgently needs the existing genetically pure specimens from other parts of the species range. Considering that the total number of grayling specimens in Soča River is low, we strongly believe that a long-term restocking program with a sufficient amount of genetically pure specimens (specifically bred for wild release) can save the original grayling of Soča river basin.